```
=> file hcaplus; d que l7; d que l10
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FILE COVERS 1907 - 12 Dec 2003  VOL 139 ISS 25
FILE LAST UPDATED: 11 Dec 2003  (20031211/ED)
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L3        13457 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ("VIRULENCE (MICROBIAL)"/CT
                OR "MICROBIAL VIRULENCE"/CT OR "MICROORGANISM VIRULENCE"/CT OR
                VIRULENCE/CT)
L4        32780 SEA FILE=HCAPLUS ABB=ON  PLU=ON  STAPHYLOCOCCUS/CW
L5           11 SEA FILE=HCAPLUS ABB=ON  PLU=ON  SARR
L7            5 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 AND L4 AND L5


L3        13457 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ("VIRULENCE (MICROBIAL)"/CT
                OR "MICROBIAL VIRULENCE"/CT OR "MICROORGANISM VIRULENCE"/CT OR
                VIRULENCE/CT)
L4        32780 SEA FILE=HCAPLUS ABB=ON  PLU=ON  STAPHYLOCOCCUS/CW
L9           14 SEA FILE=HCAPLUS ABB=ON  PLU=ON  SARA (5A) (PROMOTER OR P1)
L10           2 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3 AND L4 AND L9


=> s l7 or l10
L40           5 L7 OR L10

=> file medline; d que l18
FILE 'MEDLINE' ENTERED AT 17:14:45 ON 12 DEC 2003

 FILE LAST UPDATED: 2 DEC 2003 (20031202/UP).  FILE COVERS 1958 TO DATE.

 On April 13, 2003, MEDLINE was reloaded.  See HELP RLOAD for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2003 vocabulary.  See http://www.nlm.nih.gov/mesh/changes2003.html
 for a description on changes.

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L11       24105 SEA FILE=MEDLINE ABB=ON  PLU=ON  STAPHYLOCOCCUS AUREUS/CT
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L12       74639 SEA FILE=MEDLINE ABB=ON  PLU=ON  VIRULENCE/CT OR VIRULENCE
                FACTORS/CT
L15         912 SEA FILE=MEDLINE ABB=ON  PLU=ON  L11/MAJ (L) PY/CT
L17          50 SEA FILE=MEDLINE ABB=ON  PLU=ON  SARA PROTEIN, BACTERIAL/CN
L18          24 SEA FILE=MEDLINE ABB=ON  PLU=ON  L15 AND L12 AND L17


=> file embase; d que l24; d que l26
FILE 'EMBASE' ENTERED AT 17:14:55 ON 12 DEC 2003
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 FILE COVERS 1974 TO 11 Dec 2003 (20031211/ED)

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L19       35412 SEA FILE=EMBASE ABB=ON  PLU=ON  STAPHYLOCOCCUS AUREUS/CT
L22          11 SEA FILE=EMBASE ABB=ON  PLU=ON  SARR
L24           5 SEA FILE=EMBASE ABB=ON  PLU=ON  L19 AND L22



L19       35412 SEA FILE=EMBASE ABB=ON  PLU=ON  STAPHYLOCOCCUS AUREUS/CT
L22          11 SEA FILE=EMBASE ABB=ON  PLU=ON  SARR
L24           5 SEA FILE=EMBASE ABB=ON  PLU=ON  L19 AND L22
L26           5 SEA FILE=EMBASE ABB=ON  PLU=ON  L19 AND L24


=> s l24 or l26
L41           5 L24 OR L26

=> file biosis; d que l32; d que l33
FILE 'BIOSIS' ENTERED AT 17:15:14 ON 12 DEC 2003
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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 10 December 2003 (20031210/ED)

FILE RELOADED: 19 October 2003.


L28       58957 SEA FILE=BIOSIS ABB=ON  PLU=ON  STAPHYLOCOCCUS AUREUS
L29       44365 SEA FILE=BIOSIS ABB=ON  PLU=ON  VIRULEN?
L30          12 SEA FILE=BIOSIS ABB=ON  PLU=ON  SARR
L32           5 SEA FILE=BIOSIS ABB=ON  PLU=ON  L28 AND L29 AND L30



L20           2 SEA FILE=EMBASE ABB=ON  PLU=ON  SARR PROTEIN/CT
L28       58957 SEA FILE=BIOSIS ABB=ON  PLU=ON  STAPHYLOCOCCUS AUREUS
L31         313 SEA FILE=BIOSIS ABB=ON  PLU=ON  SARA
L33           3 SEA FILE=BIOSIS ABB=ON  PLU=ON  L28 AND L20 AND L31


=> s l32 or l33
```

```
L42           5 L32 OR L33

=> file wpid; d que l39
FILE 'WPIDS' ENTERED AT 17:15:28 ON 12 DEC 2003
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FILE LAST UPDATED:          11 DEC 2003      <20031211/UP>
MOST RECENT DERWENT UPDATE:     200380       <200380/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE


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L35        3687 SEA FILE=WPIDS ABB=ON  PLU=ON  STAPHYLOCOCC? AUREUS
L36        1341 SEA FILE=WPIDS ABB=ON  PLU=ON  VIRULEN?
L37           3 SEA FILE=WPIDS ABB=ON  PLU=ON  SARR
L38          19 SEA FILE=WPIDS ABB=ON  PLU=ON  SARA
L39           2 SEA FILE=WPIDS ABB=ON  PLU=ON  L35 AND L36 AND (L37 OR L38)


=> dup rem l18 l40 l41 l42 l39
FILE 'MEDLINE' ENTERED AT 17:15:48 ON 12 DEC 2003

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PROCESSING COMPLETED FOR L18
PROCESSING COMPLETED FOR L40
PROCESSING COMPLETED FOR L41
PROCESSING COMPLETED FOR L42
PROCESSING COMPLETED FOR L39
L43          32 DUP REM L18 L40 L41 L42 L39 (9 DUPLICATES REMOVED)
                ANSWERS '1-24' FROM FILE MEDLINE
                ANSWERS '25-28' FROM FILE HCAPLUS
                ANSWERS '29-31' FROM FILE EMBASE
                ANSWER '32' FROM FILE WPIDS
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=> d ibib ab l43 1-32

L43 ANSWER 1 OF 32 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002384905 MEDLINE
DOCUMENT NUMBER: 22128668 PubMed ID: 12133812
TITLE: Global regulation of virulence determinants in Staphylococcus aureus by the SarA protein family.
AUTHOR: Cheung Ambrose L; Zhang Gongyi
CORPORATE SOURCE: Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH 03755, USA..
ambrose.cheung@dartmouth.edu
CONTRACT NUMBER: AI37142 (NIAID)
AI50678 (NIAID)
SOURCE: Front Biosci, (2002 Aug 1) 7 d1825-42. Ref: 122
Journal code: 9709506. ISSN: 1093-4715.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 20020723
Last Updated on STN: 20021011
Entered Medline: 20021010

AB In S. aureus, the production of virulence determinants such as cell wall adhesins and exotoxins during the growth cycle is controlled by global regulators such as SarA and agr. Genomic scan reveals 16 two-component regulatory systems (e.g. agr and sae) as well as a family of SarA homologs in S. aureus. We call the SarA homologs the SarA protein family. Many of the members in this protein family are either small basic proteins (<153 residues) or two-domain proteins in which a single domain shares sequence similarity to each of the small basic proteins. Recent crystal structures of SarR and SarA reveal dimeric structures for these proteins. Because of its structure and unique mode of DNA binding, SarR, and possibly other SarA family members, may belong to a new functional class of the winged-helix family, accommodating long stretch of DNA with bending points. Based on sequence homology, we hypothesize that the SarA protein family may entail homologous structures with similar DNA-binding motifs but divergent activation domains. An understanding of how these regulators interact with each other in vivo and how they sense environmental signals to control virulence gene expression (e.g. alpha-hemolysin) will be important to our eventual goal of disrupting the regulatory network.

L43 ANSWER 2 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2003395872 MEDLINE
DOCUMENT NUMBER: 22814083 PubMed ID: 12933857
TITLE: SarT influences sarS expression in Staphylococcus aureus.
AUTHOR: Schmidt Katherine A; Manna Adhar C; Cheung Ambrose L
CORPORATE SOURCE: Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA..
Katherine.A.Schmidt@Dartmouth.edu
CONTRACT NUMBER: AI07519-14 (NIAID)
AI37142 (NIAID)
AI43968 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (2003 Sep) 71 (9) 5139-48.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 20030823
Last Updated on STN: 20030930
Entered Medline: 20030929

AB Staphylococcus aureus is a gram-positive pathogen that is capable of expressing a variety of virulence proteins in response to environmental signals. Virulence protein expression in S. aureus is controlled by a network of regulatory loci including sarA and agr. The sarA/agr network is associated with the expression of cell wall-associated adhesins during exponential growth and the expression of secreted enzymes and toxins in the transition to post-exponential growth. A number of sarA homologs, including sarT and sarS, have been identified in the S. aureus genome. Previous studies have shown that sarA influences expression of both sarT and sarS in the global regulatory network. SarS has been shown to bind to the spa promoter to induce expression of protein A. SarT, one of the SarA homologs that represses hla expression and is repressible by SarA and agr, was found to induce sarS expression in this report. Northern blot analysis of sarS and spa expression in S. aureus RN6390, and the isogenic sarT, sarT sarA, and sarT agr mutants showed that while sarA regulated spa expression directly, the agr locus used sarT as an intermediary to regulate sarS, thus leading to spa repression in agr-activated cells. Gel shift and footprinting analysis showed that SarT binds to the sarS promoter, indicating that the interaction of the sarT gene product with the upstream region of sarS is likely direct. Induction of sarS and spa by SarT in agr(+) strains was confirmed by a tetracycline-inducible system to titrate sarT expression.

L43 ANSWER 3 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2002733771 MEDLINE
DOCUMENT NUMBER: 22384175 PubMed ID: 12496203
TITLE: Role of sarA in the pathogenesis of Staphylococcus aureus musculoskeletal infection.
AUTHOR: Blevins Jon S; Elasri Mohamed O; Allmendinger Scott D; Beenken Karen E; Skinner Robert A; Thomas J Roby; Smeltzer Mark S
CORPORATE SOURCE: Department of Microbiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, USA.
CONTRACT NUMBER: AI43356 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (2003 Jan) 71 (1) 516-23.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021227
Last Updated on STN: 20030211
Entered Medline: 20030210

AB We recently demonstrated that mutation of sarA in clinical isolates of Staphylococcus aureus results in a phenotype that is distinct by comparison to sarA mutants generated in the laboratory strain RN6390 (J. S. Blevins, K. E. Beenken, M. O. Elasri, B. K. Hurlburt, and M. S. Smeltzer, Infect. Immun. 70:470-480, 2002). This raises the possibility that studies demonstrating that RN6390 sarA mutants are attenuated do not accurately reflect the role of sarA in the pathogenesis of staphylococcal disease. To test this hypothesis, we used a murine model of musculoskeletal infection to assess the virulence of sarA and agr mutants generated in a clinical isolate of S. aureus (UAMS-1). By using this model, we confirmed that mutation of sarA and/or agr results in a reduced capacity to cause both septic arthritis and osteomyelitis.

L43 ANSWER 4 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2002733756 MEDLINE
DOCUMENT NUMBER: 22384156 PubMed ID: 12496184
TITLE: sarU, a sarA homolog, is repressed by SarT and regulates virulence genes in Staphylococcus aureus.
AUTHOR: Manna Adhar C; Cheung Ambrose L
CORPORATE SOURCE: Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755, USA..
Adhar.C.Manna@Dartmouth.EDU
CONTRACT NUMBER: AI37142 (NIAID)
AI50678 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (2003 Jan) 71 (1) 343-53.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021227
Last Updated on STN: 20030408
Entered Medline: 20030210

AB In searching the Staphylococcus aureus genome, we previously identified sarT, a homolog of sarA, which encodes a repressor for alpha-hemolysin synthesis. Adjacent but transcribed divergently to sarT is sarU, which encodes a 247-residue polypeptide, almost twice the length of SarA. Sequence alignment disclosed that SarU, like SarS, which is another SarA homolog, could be envisioned as a molecule with two halves, with each half being homologous to SarA. SarU, as a member of the SarA family proteins, disclosed conservation of basic residues within the helix-turn-helix motif and within the beta hairpin loop, two putative DNA binding domains within this protein family. The transcription of sarU is increased in a sarT mutant. Gel shift and transcriptional fusion studies revealed that SarT can bind to the sarU promoter region, probably acting as a repressor for sarU transcription. The expression of RNAII and RNAIII of agr is decreased in a sarU mutant. As RNAIII expression is up-regulated in a sarT mutant, we hypothesize that sarT may down regulate agr RNAIII expression by repressing sarU, a positive activator of agr expression. We propose that, in addition to the quorum sensing effect of the autoinducing peptide of agr, the sarT-sarU pathway may represent a secondary amplification loop whereby the expression of agr (e.g., those found in vivo) might repress sarT, leading to increased expression of sarU. Elevated sarU expression would result in additional amplification of the original agr signal.

L43 ANSWER 5 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2002622045 MEDLINE
DOCUMENT NUMBER: 22267135 PubMed ID: 12379717
TITLE: Staphylococcus aureus aconitase inactivation unexpectedly inhibits post-exponential-phase growth and enhances stationary-phase survival.
AUTHOR: Somerville Greg A; Chaussee Michael S; Morgan Carrie I; Fitzgerald J Ross; Dorward David W; Reitzer Lawrence J; Musser James M
CORPORATE SOURCE: Laboratory of Human Bacterial Pathogenesis. Rocky Mountain Microscopy Branch. Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana 59840, USA.
SOURCE: INFECTION AND IMMUNITY, (2002 Nov) 70 (11) 6373-82.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021017
Last Updated on STN: 20021213
Entered Medline: 20021108

AB Staphylococcus aureus preferentially catabolizes glucose, generating pyruvate, which is subsequently oxidized to acetate under aerobic growth conditions. Catabolite repression of the tricarboxylic acid (TCA) cycle results in the accumulation of acetate. TCA cycle derepression coincides with exit from the exponential growth phase, the onset of acetate catabolism, and the maximal expression of secreted virulence factors. These data suggest that carbon and energy for post-exponential-phase growth and virulence factor production are derived from the catabolism of acetate mediated by the TCA cycle. To test this hypothesis, the aconitase gene was genetically inactivated in a human isolate of S. aureus, and the effects on physiology, morphology, virulence factor production, virulence for mice, and stationary-phase survival were examined. TCA cycle inactivation prevented the post-exponential growth phase catabolism of acetate, resulting in premature entry into the stationary phase. This phenotype was accompanied by a significant reduction in the production of several virulence factors and alteration in host-pathogen interaction. Unexpectedly, aconitase inactivation enhanced stationary-phase survival relative to the wild-type strain. Aconitase is an iron-sulfur cluster-containing enzyme that is highly susceptible to oxidative inactivation. We speculate that reversible loss of the iron-sulfur cluster in wild-type organisms is a survival strategy used to circumvent oxidative stress induced during host-pathogen interactions. Taken together, these data demonstrate the importance of the TCA cycle in the life cycle of this medically important pathogen.

L43 ANSWER 6 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001699934 MEDLINE
DOCUMENT NUMBER: 21614914 PubMed ID: 11748173
TITLE: Staphylococcus aureus agr and sarA functions are required for invasive infection but not inflammatory responses in the lung.
AUTHOR: Heyer Geoffrey; Saba Shahryar; Adamo Robert; Rush William; Soong Grace; Cheung Ambrose; Prince Alice
CORPORATE SOURCE: Columbia University College of Physicians and Surgeons, New York, New York 10032, USA.
CONTRACT NUMBER: HL56194 (NHLBI)
HL60293 (NHLBI)
SOURCE: INFECTION AND IMMUNITY, (2002 Jan) 70 (1) 127-33.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011219
Last Updated on STN: 20020125
Entered Medline: 20020114

AB Staphylococcus aureus strains lacking agr- and sarA-dependent gene products or specific MSCRAMM (microbial surface components recognizing adhesive matrix molecules) adhesins were compared for the ability to activate inflammatory responses in the lung. The mutants were evaluated for virulence in a mouse model of pneumonia and by quantifying their ability to stimulate interleukin-8 (IL-8) and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression in respiratory epithelial

cells. In a neonatal mouse, only strains with intact agr and sarA loci were consistently associated with invasive, fatal pulmonary infection ($P < 0.001$) and sarA was specifically required to cause bacteremia ($P < 0.001$). The agr and/or sarA mutants were, nonetheless, fully capable of producing pneumonia and were as proficient as the wild-type strain in stimulating epithelial IL-8 expression, a polymorphonuclear leukocyte chemokine, in airway cells. In contrast, agr and especially sarA mutants induced less epithelial GM-CSF expression, and MSCRAMM mutants lacking fibronectin binding proteins or clumping factor A, a ligand for fibrinogen, were unable to stimulate epithelial GM-CSF production. The ability to induce IL-8 expression was independent of the adherence properties of intact bacteria, indicating that shed and/or secreted bacterial components activate epithelial responses. While conserved staphylococcal components such as peptidoglycan are sufficient to evoke inflammation and cause pneumonia, the agr and sarA loci of S. aureus are critical for the coordination of invasive infection of the lungs.

L43 ANSWER 7 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2002444741 MEDLINE
DOCUMENT NUMBER: 22191865 PubMed ID: 12202106
TITLE: Regulation of Staphylococcus aureus type 5 capsular polysaccharides by agr and sarA in vitro and in an experimental endocarditis model.
AUTHOR: van Wamel Willem; Xiong Yan-Qiong; Bayer Arnold S; Yeaman Michael R; Nast Cynthia C; Cheung Ambrose L
CORPORATE SOURCE: Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH 03755, USA.
CONTRACT NUMBER: R001AI-47441 (NIAID)
R01AI-39108 (NIAID)
R01AI-48031 (NIAID)
RR-13004 (NCRR)
SOURCE: MICROBIAL PATHOGENESIS, (2002 Aug) 33 (2) 73-9.
Journal code: 8606191. ISSN: 0882-4010.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020831
Last Updated on STN: 20021217
Entered Medline: 20021203

AB The expression of antiphagocytic polysaccharide capsules is an important pathogenetic step in establishing Staphylococcus aureus infections. Using a green fluorescent protein reporter gene (gfp) system, we examined the expression and genetic regulation of the cap5 promoter (capsular polysaccharide 5 genes) by two major global regulators of S. aureus (agr and sarA) in vitro and in a rabbit endocarditis model. In vitro, cap5 expression substantially increased during the post-exponential phase in parental, as well assarA mutant constructs. However, cap5 expression was greatly reduced in agr and agr/sarA double mutants. In the endocarditis model, the extent of cap5 expression in vegetations infected with the parental strain was substantially higher than that observed with the agr/sarA double mutants ($P<0.05$). Similar trends were noted in renal, but not splenic abscesses. Collectively, these data suggest that agr positively regulates cap5 expression both in vitro and in vivo, while the contribution of sarA to cap5 regulation, although modest, is readily discerned in vivo in agr minus background. In addition, the regulation ofcap5 expression by these global regulators may vary in distinct anatomic niches in vivo.

L43 ANSWER 8 OF 32 MEDLINE on STN

ACCESSION NUMBER: 2001683101 MEDLINE
DOCUMENT NUMBER: 21586266 PubMed ID: 11728861
TITLE: Are the structures of SarA and SarR similar?.
AUTHOR: Cheung A L; Zhang G
SOURCE: TRENDS IN MICROBIOLOGY, (2001 Dec) 9 (12) 570-3.
Journal code: 9310916. ISSN: 0966-842X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: News Announcement
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011203
Last Updated on STN: 20020212
Entered Medline: 20020211

L43 ANSWER 9 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001671532 MEDLINE
DOCUMENT NUMBER: 21574171 PubMed ID: 11717293
TITLE: Transcription profiling-based identification of Staphylococcus aureus genes regulated by the agr and/or sarA loci.
AUTHOR: Dunman P M; Murphy E; Haney S; Palacios D; Tucker-Kellogg G; Wu S; Brown E L; Zagursky R J; Shlaes D; Projan S J
CORPORATE SOURCE: Infectious Diseases, Wyeth-Ayerst Research, Pearl River, New York 10965, USA.
SOURCE: JOURNAL OF BACTERIOLOGY, (2001 Dec) 183 (24) 7341-53.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011122
Last Updated on STN: 20020123
Entered Medline: 20011226

AB The advent of transcription profiling technologies has provided researchers with an unprecedented ability to study biological processes. Accordingly, a custom-made Affymetrix GeneChip, constituting >86% of the Staphylococcus aureus genome, was used to identify open reading frames that are regulated by agr and/or SarA, the two best-studied regulators of the organism's virulence response. RNA extracted from wild-type cells and agr, sarA, and agr sarA mutant cells in the early-, mid-, and late-log and stationary phases of growth was analyzed. Open reading frames with transcription patterns expected of genes either up- or downregulated in an agr- and/or SarA-dependent manner were identified. Oligonucleotide microarray and Northern blot analyses confirmed that the transcription of several known virulence genes, including hla (alpha-toxin) and spa (protein A), is regulated by each effector and provided insights about the regulatory cascades involved in both alpha-hemolysin and protein A expression. Several putative virulence factors were also identified as regulated by agr and/or SarA. In addition, genes that are involved in several biological processes but which are difficult to reconcile as playing a direct role in the organism's pathogenesis also appeared to be regulated by each effector, suggesting that products of both the agr and the sarA locus are more-global transcription regulators than previously realized.

L43 ANSWER 10 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001551444 MEDLINE
DOCUMENT NUMBER: 21481968 PubMed ID: 11598065
TITLE: Diminished virulence of an alpha-toxin mutant of

Staphylococcus aureus in experimental brain abscesses.
AUTHOR: Kielian T; Cheung A; Hickey W F
CORPORATE SOURCE: Department of Pathology, Dartmouth-Hitchcock Medical Center, Dartmouth Medical School, Lebanon, New Hampshire 03756, USA.. KielianTammyL@uams.edu
CONTRACT NUMBER: NA-27321 (NASA)
NS40730 (NINDS)
SOURCE: INFECTION AND IMMUNITY, (2001 Nov) 69 (11) 6902-11.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011015
Last Updated on STN: 20020122
Entered Medline: 20011205

AB Staphylococcus aureus is one of the major etiologic agents of brain abscesses in humans, occasionally leading to focal neurological deficits and even death. The objective of the present study was to identify key virulence determinants contributing to the pathogenesis of S. aureus in the brain using a murine brain abscess model. The importance of virulence factor production in disease development was demonstrated by the inability of heat-inactivated S. aureus to induce proinflammatory cytokine or chemokine expression or brain abscess formation in vivo. To directly address the contribution of virulence determinants in brain abscess development, the abilities of S. aureus strains with mutations in the global regulatory loci sarA and agr were examined. An S. aureus sarA agr double mutant exhibited reduced virulence in vivo, as demonstrated by attenuated proinflammatory cytokine and chemokine expression and bacterial replication. Subsequent studies focused on the expression of factors that are altered in the sarA agr double mutant. Evaluation of an alpha-toxin mutant revealed a phenotype similar to that of the sarA agr mutant in vivo, as evidenced by lower bacterial burdens and attenuation of cytokine and chemokine expression in the brain. This suggested that alpha-toxin is a central virulence determinant in brain abscess development. Another virulence mechanism utilized by staphylococci is intracellular survival. Cells recovered from brain abscesses were shown to harbor S. aureus intracellularly, providing a means by which the organism may establish chronic infections in the brain. Together, these data identify alpha-toxin as a key virulence determinant for the survival of S. aureus in the brain.

L43 ANSWER 11 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001389365 MEDLINE
DOCUMENT NUMBER: 21337016 PubMed ID: 11442841
TITLE: Impact of the regulatory loci agr, sarA and sae of Staphylococcus aureus on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis.
AUTHOR: Goerke C; Fluckiger U; Steinhuber A; Zimmerli W; Wolz C
CORPORATE SOURCE: Institute for General and Environmental Hygiene, University of Tubingen, Wilhelmstrasse 31, 72074 Tubingen, Germany.. christiane.wolz@uni-tuebingen.de
SOURCE: MOLECULAR MICROBIOLOGY, (2001 Jun) 40 (6) 1439-47.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109

ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20030325
Entered Medline: 20010906

AB The cytotoxic alpha-toxin (encoded by hla) of Staphylococcus aureus is regulated by three loci, agr, sarA and sae, in vitro. Here, we assess the regulation of hla in a guinea pig model of device-related infection by quantifying RNAIII (the effector molecule of agr) and hla directly in exudates accumulating in infected devices without subculturing of the bacteria. LightCycler reverse transcription-polymerase chain reaction (RT-PCR) was used to quantify the transcripts. Strains RN6390 and Newman expressed considerably smaller amounts of RNAIII in the guinea pig than during in vitro growth. The residual RNAIII expression decreased during the course of infection and was negatively correlated with bacterial densities. As with RNAIII, the highest hla expression was detected in both strains early in infection. Even in strain Newman, a weak hla producer in vitro, a pronounced expression of hla was observed during infection. Likewise, four S. aureus isolates from cystic fibrosis (CF) patients expressed Q1hla despite an inactive agr during device-related infection as in the CF lung. Mutation of agr and sarA in strain Newman and RN6390 had no consequence for hla expression in vivo. In contrast, the mutation in sae resulted in severe downregulation of hla in vitro as well as in vivo. In conclusion, S. aureus seems to be provided with regulatory circuits different from those characterized in vitro to ensure alpha-toxin synthesis during infections.

L43 ANSWER 12 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001573267 MEDLINE
DOCUMENT NUMBER: 21538277 PubMed ID: 11681202
TITLE: Extracellular proteins of Staphylococcus aureus and the role of SarA and sigma B.
AUTHOR: Ziebandt A K; Weber H; Rudolph J; Schmid R; Hoper D; Engelmann S; Hecker M
CORPORATE SOURCE: Institut fur Mikrobiologie und Molekularbiologie, Jahnstr. 15, D-17487, Greifswald, Germany.
SOURCE: Proteomics, (2001 Apr) 1 (4) 480-93.
Journal code: 101092707. ISSN: 1615-9853.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011030
Last Updated on STN: 20020123
Entered Medline: 20011218

AB Staphylococcus aureus synthesizes a large number of extracellular proteins that have been postulated to play a role in bacterial virulence. The proteomic approach was used to analyse the pattern of extracellular proteins of two different S. aureus strains, RN6390 and COL. Thirty-nine protein spots were identified by N-terminal sequencing or MALDI-TOF-MS. The differences of the extracellular protein patterns between both strains are striking. Among the 18 proteins identified in S. aureus COL there are nine proteins not yet discovered in S. aureus RN6390. These are enterotoxin B, leukotoxin D, enterotoxin, serin proteases (SplA and SplC), thermonuclease, an IgG binding protein and two so far unknown proteins in S. aureus with similarities to SceD precursor in Staphylococcus carnosus and to synergohymenotropic toxin precursor in Streptococcus intermedius. In contrast, lipase as well as staphylokinase identified in S. aureus RN6390 were not detectable in S. aureus COL under the same conditions. By using a regulatory mutant of sarA (ALC136) isogenic to strain RN6390 we identified five proteins positively regulated by SarA and 12 proteins negatively regulated by SarA. Besides V8 protease (StsP) and Hlb already

described to be regulated by the sar locus new putatively sarA-dependent proteins were identified, e.g. glycerolester hydrolase and autolysin both down-regulated in the sarA mutant, and aureolysin, staphylokinase, staphopain and format tetrahydrofolate lyase up-regulated in the mutant. Moreover, the role of sigma B in expression of extracellular proteins was studied. Interestingly, we found 11 proteins at an enhanced level in a sigB mutant of S. aureus COL, among them enterotoxin B, alpha and beta hemolysin, serine proteases SplA and SplB, leukotoxin D, and staphopain homologues. The sigma B-dependent repression of gene expression occurs at the transcriptional level. Only one protein, SceD, was identified whose synthesis was down-regulated in the mutant indicating that its gene belongs to the sigma B-dependent general stress regulon.

L43 ANSWER 13 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2001100888 MEDLINE
DOCUMENT NUMBER: 21037969 PubMed ID: 11196648
TITLE: Crystal structures of SarA, a pleiotropic regulator of virulence genes in S. aureus.
COMMENT: Erratum in: Nature 2001 Nov 1;414(6859):85
AUTHOR: Schumacher M A; Hurlburt B K; Brennan R G
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland 97201-3098, USA.
SOURCE: NATURE, (2001 Jan 11) 409 (6817) 215-9.
Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1FZN; PDB-1FZP
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010201

AB Staphylococcus aureus is a major human pathogen, the potency of which can be attributed to the regulated expression of an impressive array of virulence determinants. A key pleiotropic transcriptional regulator of these virulence factors is SarA, which is encoded by the sar (staphylococcal accessory regulator) locus. SarA was characterized initially as an activator of a second virulence regulatory locus, agr, through its interaction with a series of heptad repeats (AGTTAAG) within the agr promoter. Subsequent DNA-binding studies have revealed that SarA binds readily to multiple AT-rich sequences of variable lengths. Here we describe the crystal structure of SarA and a SarA-DNA complex at resolutions of 2.50 A and 2.95 A, respectively. SarA has a fold consisting of a four-helix core region and 'inducible regions' comprising a beta-hairpin and a carboxy-terminal loop. On binding DNA, the inducible regions undergo marked conformational changes, becoming part of extended and distorted alpha-helices, which encase the DNA. SarA recognizes an AT-rich site in which the DNA is highly overwound and adopts a D-DNA-like conformation by indirect readout. These structures thus provide insight into SarA-mediated transcription regulation.

L43 ANSWER 14 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2000192031 MEDLINE
DOCUMENT NUMBER: 20192031 PubMed ID: 10725730
TITLE: Survival of Staphylococcus aureus inside neutrophils contributes to infection.
AUTHOR: Gresham H D; Lowrance J H; Caver T E; Wilson B S; Cheung A L; Lindberg F P
CORPORATE SOURCE: Research Service, Albuquerque Veterans Affairs Medical Center, Albuquerque, NM, 87108, USA..

hgersham@salud.unm.edu
CONTRACT NUMBER: AI 30061 (NIAID)
GM 57573 (NIGMS)
SOURCE: JOURNAL OF IMMUNOLOGY, (2000 Apr 1) 164 (7) 3713-22.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000512
Last Updated on STN: 20020727
Entered Medline: 20000504

AB Neutrophils have long been regarded as essential for host defense against Staphylococcus aureus infection. However, survival of the pathogen inside various cells, including phagocytes, has been proposed as a mechanism for persistence of this microorganism in certain infections. Therefore, we investigated whether survival of the pathogen inside polymorphonuclear neutrophils (PMN) contributes to the pathogenesis of S. aureus infection. Our data demonstrate that PMN isolated from the site of infection contain viable intracellular organisms and that these infected PMN are sufficient to establish infection in a naive animal. In addition, we show that limiting, but not ablating, PMN migration into the site of infection enhances host defense and that repletion of PMN, as well as promoting PMN influx by CXC chemokine administration, leads to decreased survival of the mice and an increased bacterial burden. Moreover, a global regulator mutant of S. aureus (sar-) that lacks the expression of several virulence factors is less able to survive and/or avoid clearance in the presence of PMN. These data suggest that the ability of S. aureus to exploit the inflammatory response of the host by surviving inside PMN is a virulence mechanism for this pathogen and that modulation of the inflammatory response is sufficient to significantly alter morbidity and mortality induced by S. aureus infection.

L43 ANSWER 15 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2000497193 MEDLINE
DOCUMENT NUMBER: 20392468 PubMed ID: 10931334
TITLE: Identification and characterization of SarH1, a new global regulator of virulence gene expression in Staphylococcus aureus.
AUTHOR: Tegmark K; Karlsson A; Arvidson S
CORPORATE SOURCE: Microbiology and Tumorbiology Center (MTC), Box 280, Karolinska Institutet, S-17177 Stockholm, Sweden.
SOURCE: MOLECULAR MICROBIOLOGY, (2000 Jul) 37 (2) 398-409.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001018

AB The global regulators agr (accessory gene regulator) and sarA (staphylococcal accessory regulator) have been reported to be both activators and repressors of virulence gene expression in Staphylococcus aureus. How the effector of the agr system, RNAIII, interacts with target gene promoters is unknown. SarA, on the other hand, is a DNA-binding protein, which binds to conserved DNA motifs immediately upstream of both positively and negatively regulated promoters. Here, we searched for additional regulators that could explain the differential effects of

RNAIII and SarA. Four differently regulated genes (hla, alpha-toxin; hld, RNAIII; spa, protein A; ssp, serine protease) were analysed for binding of potential regulatory proteins to the corresponding promoter DNA fragments, linked to magnetic beads. One protein (29 kDa), with affinity for all four promoters, showed a high degree of similarity to SarA and was named SarH1 (Sar homologue 1). Expression of sarH1 was strongly repressed by sarA and agr. Analysis of hla, hld, ssp and spa mRNAs in sarH1, sarA and agr mutants, and in sarA/sarH1 and agr/sarH1 double mutants, revealed that sarH1 has a strong repressive effect on hla and an activating effect on spa transcription. SDS-PAGE analysis of secreted proteins from the different mutants showed that the production of several other exoproteins was affected by sarH1. In conclusion, we show that both the agr-dependent suppression of protein A production and the sarA-dependent stimulation of alpha-toxin production is mediated via a new regulator, SarH1, which belongs to a family of Sar homologues.

L43 ANSWER 16 OF 32 MEDLINE on STN
ACCESSION NUMBER: 2000223665 MEDLINE
DOCUMENT NUMBER: 20223665 PubMed ID: 10760180
TITLE: Agr-independent regulation of fibronectin-binding protein(s) by the regulatory locus sar in Staphylococcus aureus.
AUTHOR: Wolz C; Pohlmann-Dietze P; Steinhuber A; Chien Y T; Manna A; van Wamel W; Cheung A
CORPORATE SOURCE: The Laboratory of Bacterial Pathogenesis and Immunology, the Rockefeller University, New York, NY 10021, USA.. christiane.wolz@uni-tuebingen.de
CONTRACT NUMBER: AI37142 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (2000 Apr) 36 (1) 230-43. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000606
Last Updated on STN: 20000606
Entered Medline: 20000523

AB Fibronectin-binding proteins (FnBPs) are thought to be important for the attachment of Staphylococcus aureus during infection. The regulation of the genes fnbA and fnbB by the global regulatory loci sar and agr was examined using site-specific regulatory mutants of S. aureus strain Newman. The results from binding assays using both aqueous and solid-phase fibronectin as well as ligand blotting with biotinylated fibronectin showed that the expression of FnBPA is enhanced in the agr mutant but inhibited in the sar mutant and the sar-agr double mutant. The same regulatory pattern was observed in Northern blot analysis using fnbA-specific probes. The introduction of sar on a multicopy plasmid increased the already enhanced fnbA transcription of the agr mutant. FnBPB was not detectable by ligand blotting and the fnbB promoter activity in promoter fusion assays was not affected by either sar or agr. The sequence encompassing ORF3 located upstream of sarA was found to be essential for the activation of fnbA transcription. We hypothesize that this sequence may modulate SarA expression and/or activity on the post-transcriptional level. Gel shift assays demonstrated that SarA binds to the fnbA promoter fragments, probably as a dimer. DNase I footprinting assays with SarA revealed a protected area of 102 bp upstream of fnbA.

L43 ANSWER 17 OF 32 MEDLINE on STN
ACCESSION NUMBER: 1999444917 MEDLINE
DOCUMENT NUMBER: 99444917 PubMed ID: 10517329

TITLE: Interactive regulatory pathways control virulence determinant production and stability in response to environmental conditions in Staphylococcus aureus.
AUTHOR: Lindsay J A; Foster S J
CORPORATE SOURCE: Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, UK.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1999 Sep) 262 (2) 323-31. Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991101

AB The accessory gene regulator (agr) and staphylococcal accessory regulator (sar) loci are important regulators of toxin production in Staphylococcus aureus. In this study we examined how environmental conditions degree of aeration and salt concentration - affect the transcription and translation of mRNAs for alpha-haemolysin (Hla) and serine protease (Ssp) via these pathways and influence the stability of these proteins. Using Northern analysis, we have confirmed earlier observations that sarA is involved in the upregulation of RNAIII, the effector molecule encoded by the agr locus. However, this effect was abolished in highly aerated cultures. While sarA does appear to have an up-regulatory effect on hla transcription that is independent of agr, we propose that the PC1839 (sarA) mutant produces less alpha-haemolysin activity mainly as a result of post-translational inactivation by proteases. The most obvious phenotypic feature of PC1839 (sarA) is the upregulation of proteases. In this study we show that ssp is repressed by SarA at the transcriptional level. Western analysis using an anti-alpha-haemolysin antibody identified a major breakdown product that is only present in the supernatant of strains that are overexpressing serine protease. We have also confirmed that agr exerts a significant regulatory influence on hla at the level of translation, as well as transcription. Finally, the addition of salt upregulates ssp transcription and dramatically downregulates transcription of hla, and is an example of an environmental parameter that affects toxin production independently of agr and sarA. How environmental signals are transduced to control alpha-haemolysin and serine protease production, activity and stability at multiple levels are discussed.

L43 ANSWER 18 OF 32 MEDLINE on STN
ACCESSION NUMBER: 1999340210 MEDLINE
DOCUMENT NUMBER: 99340210 PubMed ID: 10411747
TITLE: Characterization of the SarA virulence gene regulator of Staphylococcus aureus.
AUTHOR: Rechtin T M; Gillaspy A F; Schumacher M A; Brennan R G; Smeltzer M S; Hurlburt B K
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock 72205, USA.
CONTRACT NUMBER: AI43356 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Jul) 33 (2) 307-16. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909

ENTRY DATE: Entered STN: 19990913
Last Updated on STN: 19990913
Entered Medline: 19990902

AB Staphylococcus aureus is a potent human pathogen that expresses a large number of virulence factors in a temporally regulated fashion. Two pleiotropically acting regulatory loci were identified in previous mutational studies. The agr locus comprises two operons that express a quorum-sensing system from the P2 promoter and a regulatory RNA molecule from the P3 promoter. The sar locus encodes a DNA-binding protein that activates the expression of both agr operons. We have cloned the sarA gene, expressed SarA in Escherichia coli and purified the recombinant protein to apparent homogeneity. The purified protein was found to be dimeric in the presence and absence of DNA and to consist mostly of alpha-helices. DNase I footprinting of SarA on the putative regulatory region cis to the agr promoters revealed three high-affinity binding sites composed of two half-sites each. Quantitative electrophoretic mobility shift assays (EMSAs) were used to derive equilibrium binding constants (KD) for the interaction of SarA with these binding sites. An unusual ladder banding pattern was observed in EMSA with a large DNA fragment including all three binding sites. Our data indicate that SarA regulation of the agr operons involves binding to multiple half-sites and may involve other sites located downstream of the promoters.

L43 ANSWER 19 OF 32 MEDLINE on STN
ACCESSION NUMBER: 1999047569 MEDLINE
DOCUMENT NUMBER: 99047569 PubMed ID: 9829932
TITLE: Role of SarA in virulence determinant production and environmental signal transduction in Staphylococcus aureus.
AUTHOR: Chan P F; Foster S J
CORPORATE SOURCE: Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, United Kingdom.
SOURCE: JOURNAL OF BACTERIOLOGY, (1998 Dec) 180 (23) 6232-41.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303
Entered Medline: 19981224

AB The staphylococcal accessory regulator (encoded by sarA) is an important global regulator of virulence factor biosynthesis in Staphylococcus aureus. To further characterize its role in virulence determinant production, an sarA knockout mutant was created by insertion of a kanamycin antibiotic resistance cassette into the sarA gene. N-terminal sequencing of exoproteins down-regulated by sarA identified several putative proteases, including a V8 serine protease and a novel metalloprotease, as the major extracellular proteins repressed by sarA. In kinetic studies, the sarA mutation delays the onset of alpha-hemolysin (encoded by hla) expression and reduces levels of hla to approximately 40% of the parent strain level. Furthermore, SarA plays a role in signal transduction in response to microaerobic growth since levels of hla were much lower in a microaerobic environment than after aerobic growth in the sarA mutant. An exoprotein exhibiting hemolysin activity on sheep blood, and up-regulated by sarA independently of the accessory gene regulator (encoded by agr), was specifically induced microaerobically. Transcriptional gene fusion and Western analysis revealed that sarA up-regulates both toxic shock syndrome toxin 1 gene (tst) expression and staphylococcal enterotoxin B production, respectively. This study demonstrates the role of sarA as a signal transduction regulatory

component in response to aeration stimuli and suggests that sarA functions as a major repressor of protease activity. The possible role of proteases as regulators of virulence determinant stability is discussed.

L43 ANSWER 20 OF 32 MEDLINE on STN
ACCESSION NUMBER: 1999003134 MEDLINE
DOCUMENT NUMBER: 99003134 PubMed ID: 9784528
TITLE: Staphylococcus aureus Agr and Sar global regulators influence internalization and induction of apoptosis.
AUTHOR: Wesson C A; Liou L E; Todd K M; Bohach G A; Trumble W R; Bayles K W
CORPORATE SOURCE: Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, Idaho 83844-3052, USA.
CONTRACT NUMBER: AI28401 (NIAID)
R29-AI38901 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1998 Nov) 66 (11) 5238-43.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123

AB Staphylococcus aureus was recently shown to be internalized by and to induce apoptosis in a bovine mammary epithelial cell line, suggesting that these processes could be involved in staphylococcal pathogenesis or persistence. To examine the role of virulence factor regulators during internalization, mutant agr and sar strains of S. aureus were analyzed for their abilities to enter and induce apoptosis in epithelial cells. Like a previously characterized bovine mastitis isolate, the standard laboratory strain, RN6390 (wild type), entered the epithelial cells and subsequently induced apoptosis. In contrast, the mutant strains RN6911 (agr), ALC136 (sar), and ALC135 (agr sar) were internalized by the cultured cells at levels reproducibly greater than that for RN6390 but failed to induce apoptosis. The internalization of S. aureus was affected by growth phase, suggesting a role for agr-regulated surface proteins in this process. Furthermore, the ability to induce apoptosis required metabolically active intracellular bacteria. These data indicate that the ability of S. aureus to enter mammalian cells and induce apoptosis is dependent on factors regulated by Agr and Sar. Since transcriptional control by these global regulators is mediated by quorum-sensing and environmental factors, staphylococci may have the potential to induce several alternative effects on cells from an intracellular environment. A model for the function of the agr locus in the context of internalization, intracellular persistence, and dissemination is proposed.

L43 ANSWER 21 OF 32 MEDLINE on STN
ACCESSION NUMBER: 1998112807 MEDLINE
DOCUMENT NUMBER: 98112807 PubMed ID: 9446568
TITLE: Molecular interactions between two global regulators, sar and agr, in Staphylococcus aureus.
AUTHOR: Chien Y; Cheung A L
CORPORATE SOURCE: Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, New York 10021, USA.
CONTRACT NUMBER: AI30061 (NIAID)
AI37142 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jan 30) 273 (5) 2645-52.

Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980306
Last Updated on STN: 19980306
Entered Medline: 19980223

AB The expression of many virulence determinants in Staphylococcus aureus is controlled by regulatory loci such as agr and sar. We have previously shown that the SarA protein is required for optimal transcription of RNAII and RNAIII in the agr locus. To define the specific molecular interaction, we overexpressed SarA as a glutathione S-transferase (GST) fusion protein by cloning the 372-base pair (bp) sarA gene into the vector. The purified GST-SarA as well as cleaved SarA were able to bind specifically to the P2, P3, and the combined P2-P3 promoter fragments of agr in gel shift assays. Using monoclonal antibodies to SarA, we found that SarA is a part of the retarded protein-DNA complex as evidenced by the formation of a supershifted band. The SarA binding site on the agr promoter, mapped by DNase I footprinting assay, covered a 29-bp region between the P2 and P3 promoters devoid of any direct repeats. A synthetic 45-bp fragment encompassing the 29-bp sequence also bound the SarA protein in band shift assays. Serial in-frame deletion analysis of sarA revealed that, with the exception of 15 residues in the N terminus, almost all of SarA (residues 16-124) is essential for agr binding activity. Northern analysis confirmed that only the sar mutant clone containing a truncated sarA gene with a 15-residue deletion in the N terminus (SarA16-124) could activate agr transcription to a level approaching that of the full-length counterpart (SarA1-124). Taken together, these data indicated that SarA is a DNA-binding protein with binding specificity to the P2 and P3 interpromoter region of agr, thereby activating RNAII and RNAIII transcription.

L43 ANSWER 22 OF 32 MEDLINE on STN
ACCESSION NUMBER: 97230339 MEDLINE
DOCUMENT NUMBER: 97230339 PubMed ID: 9119503
TITLE: Staphylococcal accessory regulator (sar) in conjunction with agr contributes to Staphylococcus aureus virulence in endophthalmitis.
AUTHOR: Booth M C; Cheung A L; Hatter K L; Jett B D; Callegan M C; Gilmore M S
CORPORATE SOURCE: Department of Ophthalmology and Dean A. McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City 73190, USA.. mary-booth@uokhsc.edu
CONTRACT NUMBER: EY08289 (NEI)
EY10867 (NEI)
SOURCE: INFECTION AND IMMUNITY, (1997 Apr) 65 (4) 1550-6.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 19990129
Entered Medline: 19970424

AB Previous studies showed that an agr mutant strain of Staphylococcus aureus was partially attenuated in virulence compared to a parental strain in experimental endophthalmitis. The purpose of this study was to determine whether the sar locus, either alone or through interactions with agr,

contributes to the regulation of virulence in S. aureus endophthalmitis. Experimental endophthalmitis was established by the midvitreous injection of approximately 30 CFU of S. aureus RN6390 or the isogenic attenuated strains RN6911 (agr mutant), ALC136 (sar mutant), and ALC135 (agr sar double mutant). Unexpectedly, the rate of reduction in electroretinographic B-wave amplitude in eyes infected with strain ALC136 (sar mutant) was not significantly different from the parental strain on postinfection day (PID) 5 (10% retention). In contrast, ALC135 (agr sar double mutant)-infected eyes retained 73% of preoperative B-wave amplitude on PID 5. Therefore, unlike agr, a mutation in the sar locus alone does not alter the overall virulence of wild-type S. aureus in experimental endophthalmitis. However, the combined effect of insertional mutations in both the sar and agr global regulators leads to near-complete attenuation of virulence.

L43 ANSWER 23 OF 32 MEDLINE on STN
ACCESSION NUMBER: 96345622 MEDLINE
DOCUMENT NUMBER: 96345622 PubMed ID: 8755885
TITLE: The molecular architecture of the sar locus in Staphylococcus aureus.
AUTHOR: Bayer M G; Heinrichs J H; Cheung A L
CORPORATE SOURCE: Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, 10021, USA..
CONTRACT NUMBER: AI30061 (NIAID)
AI37142 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1996 Aug) 178 (15) 4563-70.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U46541
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961008
Last Updated on STN: 19990129
Entered Medline: 19960926

AB The global regulator sar in Staphylococcus aureus controls the synthesis of a variety of cell wall and extracellular proteins, many of which are putative virulence factors. The sar locus in strain RN6390 contains a 339-bp open reading frame (sarA) and an 860-bp upstream region. Transcriptional analyses of this locus revealed three different transcripts of 0.58, 0.84, and 1.15 kb (designated sarA, sarC, and sarB, respectively). All three transcripts seemed to be under temporal, growth cycle-dependent regulation, with sarA and sarB being most abundant in early log phase and the sarC concentration being highest toward the late stationary phase. Mapping of the 5' ends of the sar transcripts by primer extension and modified S1 nuclease protection assays demonstrated that transcription is initiated from three separate, widely spaced promoters. The 3' ends of all three sar transcripts are identical, and transcriptional termination occurs upstream of a typical prokaryotic poly(T) termination signal. Northern (RNA) analysis of sar mutant clones containing plasmids that comprised various promoters and the termination signal revealed that individual transcripts can be generated from each of the three promoters, thus suggesting possible activation as independent promoters. The multipromoter system, from which transcription is initiated, bears conserved features for recognition by homologous sigma 70 transcription factors and also by those expressed in the general stress response. Downstream of the two distal promoters (P3 and P2) are two regions potentially encoding short peptides. It is conceivable that posttranslational cooperation between these short peptides and the sarA gene product occurs to modulate sar-related functions. Complementation

studies of a sar mutant with a clone expressing all three sar transcripts showed that this clone was able to restore the sar wild-type phenotype to the sar mutant.

L43 ANSWER 24 OF 32 MEDLINE on STN
ACCESSION NUMBER: 94292439 MEDLINE
DOCUMENT NUMBER: 94292439 PubMed ID: 8021198
TITLE: Cloning and sequencing of sarA of Staphylococcus aureus, a gene required for the expression of agr.
AUTHOR: Cheung A L; Projan S J
CORPORATE SOURCE: Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University, New York, New York 10021.
CONTRACT NUMBER: AI30061 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (1994 Jul) 176 (13) 4168-72. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U20782
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940815
Last Updated on STN: 19990129
Entered Medline: 19940729

AB To evaluate the effect of a sar mutation on the agr locus, Northern (RNA) blotting was performed to determine the levels of RNAIII, the agr regulatory molecule, in two isogenic pairs of Staphylococcus aureus strains. Our results demonstrated that RNAIII was either significantly diminished or absent in both sar mutants compared with the parents. The RNAIII level was partially restored in sar mutants complemented with an intact sar gene (designated sarA). Additionally, we were able to complement selected sar phenotypes with a plasmid carrying RNAIII (pRN6735). These studies suggest that the sarA gene is necessary for the optimal expression of agr. The sarA gene of strain RN450 was subsequently cloned and sequenced. Sequence analysis revealed an open reading frame of 372 bp with a predicted molecular size of 14,718 Da and a deduced pI of 8.52. The deduced protein sequence has a predominance of charged residues (33%) and shares sequence similarity with the virF gene of Shigella flexneri.

L43 ANSWER 25 OF 32 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2002:676165 HCAPLUS
DOCUMENT NUMBER: 137:213537
TITLE: The **sarR** gene of Staphylococcus aureus down-regulating genes for virulence factors
INVENTOR(S): Cheung, Ambrose L.; Manna, Adhar; Zhang, Gongyi
PATENT ASSIGNEE(S): Trustees of Dartmouth College, USA
SOURCE: PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002068610	A2	20020906	WO 2002-US877	20020111
WO 2002068610	A3	20031030		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2003114650 A1 20030619 US 2002-43539 20020111
PRIORITY APPLN. INFO.: US 2001-261233P P 20010112
US 2001-261607P P 20010112
US 2001-289601P P 20010508

AB A novel gene, **sarR**, which downregulates the expression of sarA and the resulting virulence determination in Staphylococcus aureus is provided. Methods for modulating the expression of sar A and virulence determinants are also provided. A preferred embodiment of the present invention provides structural information relating to the gene product and enables the identification and formulation of lead compds. and reducements for treating and preventing infections by S. aureus and related bacteria. The **sarR** gene product was purified by affinity chromatog. against the P2 **promoter** of the **sarA** gene. Amino acid sequence-derived primers were used to amplify a fragment of the gene that was used to probe a ClaI partial digest library. The gene was cloned and expressed in the prior art pET11 expression vector. The interactions between the protein and the sarA protein were studied in detail. Inactivation of the **sarA** gene increased expression from the **P1** and P2-P3-P1 promoters. Anal. of the crystal structure of a fusion protein of **sarR** and maltose-binding protein indicated that the function of **sarR** is more complicated than simple repression.

L43 ANSWER 26 OF 32 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 2001:76097 HCAPLUS
DOCUMENT NUMBER: 135:163267
TITLE: Characterization of **sarR**, a modulator of sar expression in Staphylococcus aureus
AUTHOR(S): Manna, Adhar; Cheung, Ambrose L.
CORPORATE SOURCE: Department of Microbiology, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: Infection and Immunity (2001), 69(2), 885-896
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The expression of virulence determinants in Staphylococcus aureus is controlled by global regulatory loci (e.g., sar and agr). The sar locus is composed of three overlapping transcripts (sar P1, P3, and P2 transcripts from P1, P3, and P2 promoters, resp.), all encoding the 372-bp sarA gene. The level of SarA, the major regulatory protein, is partially controlled by the differential activation of sar promoters. We previously partially purified a .apprx.12 kDa protein with a DNA-specific column containing a sar P2 promoter fragment. In this study, the putative gene, designated **sarR**, was identified and found to encode a 13.6-kDa protein with homol. to SarA. Transcriptional and immunoblot studies revealed the **sarR** gene to be expressed in other staphylococcal strains. Recombinant **SarR** protein bound sar P1, P2, and P3 promoter fragments in gel shift and footprinting assays. A **sarR** mutant expressed a higher level of P1 transcript than the parent, as confirmed by promoter green fluorescent protein fusion assays. As the P1 transcript is the predominant sar transcript, we confirmed that the **sarR** mutant expressed more SarA than the parental strain. We thus proposed that **SarR** is a regulatory protein that binds to the sar promoters to down-regulate **P1** transcription and the ensuing

SarA protein expression.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 27 OF 32 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2001:915312 HCAPLUS
DOCUMENT NUMBER: 136:196686
TITLE: Are the structures of SarA and **SarR** similar?
AUTHOR(S): Cheung, Ambrose L.; Zhang, Gongyi
CORPORATE SOURCE: Dep. Microbiology, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: Trends in Microbiology (2001), 9(12), 570-573
CODEN: TRMIEA; ISSN: 0966-842X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In Staphylococcus aureus, the production of virulence determinants including hemolysin is controlled by global regulators such as SarA. Recently the crystal structures of SarA and **SarR**, a SarA homolog and a member of the SarA family of proteins, were solved. A motif found in **SarR** is similar to that found in the winged-helix protein family, and it is possible that the SarA family of proteins uses DNA bending to regulate gene transcription.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L43 ANSWER 28 OF 32 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:822140 HCAPLUS
DOCUMENT NUMBER: 138:350860
TITLE: Crystal structures of SarA, a pleiotropic regulator of virulence genes in S. aureus. [Erratum to document cited in CA134:219488]
AUTHOR(S): Schumacher, Marie A.; Hurlburt, Barry K.; Brennan, Richard G.
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology and t Vollum Institute, Oregon Health Sciences University, Portland, OR, 97201-3098, USA
SOURCE: Nature (London, United Kingdom) (2001), 414(6859), 85
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In light of the x-ray structure determination of the **SarR**-maltose binding fusion protein by Zhang et al. (2001), the crystal structure detns. of the Staphylococcus aureus transcription regulator, SarA, were re-examined Although suggested homologues (27% identity), the SarA and **SarR** structures are significantly dissimilar. The structures of the apo and DNA-bound forms of SarA may harbor some anomalies. A comprehensive exam. was performed of the structure and function relationships of a global virulence gene regulator in Staphylococcus aureus, SarA. The full-length, recombinant protein, expressed in Escherichia coli and purifd. to apparent homogeneity, bound with high affinity to cis regulatory sequences upstream of virulence genes previously reported to be controlled by SarA. The SIR and MAD (as well as averaged)-derived phases that were used to calculate the SarA-DNA complex structure resulted in electron d. maps that showed consistent secondary structure features different from those of **SarR**. In addition, MAD data for one of the "apo" sarA crystals revealed similar features to the DNA-bound form of SarA. These results suggested that the mol. is highly flexible and capable of undergoing remarkable structural changes. In suport of this are the facts that there is a remarkable change in space

gorup (from P212121 to P21212) and c cell edge (from 141 Å to 27 Å) upon freezing; all cryocooled crystals were non-isomorphous; the protein becomes inactive over time and degrades; the SarA-DNA complex reveals only nonspecific contacts; and there is an unprecedented change in protein conformation upon ligand binding. Using both an in vivo assay for virulence gene regulation and an in vitro DNA-binding assay for SarA function, most of the mutants expected to result in aberrant activity had significantly altered activity (K. Sterba, M. S. Smeltzer and B. K. Hurlburt, unpublished results).

L43 ANSWER 29 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1

ACCESSION NUMBER: 2003283312 EMBASE
TITLE: Crystal structure of the SarS protein from Staphylococcus aureus.
AUTHOR: Li R.; Manna A.C.; Dai S.; Cheung A.L.; Zhang G.
CORPORATE SOURCE: G. Zhang, 1400 Jackson St. K405, Denver, CO 80206, United States. zhangg@njc.org
SOURCE: Journal of Bacteriology, (2003) 185/14 (4219-4225).
Refs: 38
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The expression of virulence determinants in Staphylococcus aureus is controlled by global regulatory loci (e.g., sarA and agr). One of these determinants, protein A (spa), is activated by sarS, which encodes a 250-residue DNA-binding protein. Genetic analysis indicated that the agr locus likely mediates spa repression by suppressing the transcription of sarS. Contrary to SarA and **SarR**, which require homodimer formation for proper function, SarS is unusual within the SarA protein family in that it contains two homologous halves, with each half sharing sequence similarity to SarA and **SarR**. Here we report the 2.2 A resolution X-ray crystal structure of the SarS protein. SarS has folds similar to those of **SarR** and, quite plausibly, the native SarA structure. Two typical winged-helix DNA-binding domains are connected by a well-ordered loop. The interactions between the two domains are extensive and conserved. The putative DNA-binding surface is highly positively charged. In contrast, negatively charged patches are located opposite to the DNA-binding surface. Furthermore, sequence alignment and structural comparison revealed that MarR has folds similar to those of **SarR** and SarS. Members of the MarR protein family have previously been implicated in the negative regulation of an efflux pump involved in multiple antibiotic resistance in many gram-negative species. We propose that MarR also belongs to the winged-helix protein family and has a similar mode of DNA binding as **SarR** and SarS and possibly the entire SarA protein family member. Based on the structural differences of **SarR**, SarS, and MarR, we further classified these winged-helix proteins to three subfamilies, SarA, SarS, and MarR. Finally, a possible transcription regulation mechanism is proposed.

L43 ANSWER 30 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4

ACCESSION NUMBER: 2001213124 EMBASE
TITLE: Crystal structure of the **sarR** protein from Staphylococcus aureus.
AUTHOR: Liu Y.; Manna A.; Li R.; Martin W.E.; Murphy R.C.; Cheung A.L.; Zhang G.
CORPORATE SOURCE: G. Zhang, 1400 Jackson Street, Denver, CO 80206, United

States. zhangg@njc.org
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (5 Jun 2001) 98/12 (6877-6882).
Refs: 35
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The expression of virulence determinants in Staphylococcus aureus is controlled by global regulatory loci (e.g., sarA and agr). The sar (Staphylococcus accessory regulator) locus is composed of three overlapping transcripts (sarA P1, P3, and P2, transcripts initiated from the P1, P3, and P2 promoters, respectively), all encoding the 124-aa Sara protein. The level of SarA, the major regulatory protein, is partially controlled by the differential activation of the sarA promoters. We previously partially purified a 13.6-kDa protein, designated **SarR**, that binds to the sarA promoter region to down-modulate sarA transcription from the P1 promoter and subsequently SarA expression. **SarR** shares sequence similarity to SarA, and another SarA homolog, SarS. Here we report the 2.3 A-resolution x-ray crystal structure of the dimeric **SarR**-MBP (maltose binding protein) fusion protein. The structure reveals that the **SarR** protein not only has a classic helix-turn-helix module for DNA binding at the major grooves, but also has an additional loop region involved in DNA recognition at the minor grooves. This interaction mode could represent a new functional class of the "winged helix" family. The dimeric **SarR** structure could accommodate an unusually long stretch of ≈27 nucleotides with two or four bending points along the course, which could lead to the bending of DNA by 90° or more, similar to that seen in the catabolite activator protein (CAP)-DNA complex. The structure also demonstrates the molecular basis for the stable dimerization of the **SarR** monomers and possible motifs for interaction with other proteins.

L43 ANSWER 31 OF 32 EMBASE
on STN
ACCESSION NUMBER: 2001258657 EMBASE
TITLE: SarT, a repressor of α-hemolysin in Staphylococcus aureus.
AUTHOR: Schmidt K.A.; Manna A.C.; Gill S.; Cheung A.L.
CORPORATE SOURCE: K.A. Schmidt, Department of Microbiology, Dartmouth Medical School, 206 Vail Bldg., Hanover, NH 13755, United States. Katherine.a.schmidt@dartmouth.edu
SOURCE: Infection and Immunity, (2001) 69/8 (4749-4758).
Refs: 45
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In searching the Staphylococcus aureus genome, we found several homologs to SarA. One of these genes, sarT, codes for a basic protein with 118 residues and a predicted molecular size of 16,096 Da. Northern blot analysis revealed that the expression of sarT was repressed by sarA and agr. An insertion sarT mutant generated in S. aureus RN6390 and 8325-4 backgrounds revealed minimal effect on the expression of **sarR** and sarA. The RNAIII level was notably increased in the sarT mutant, particularly in postexponential-phase cells, while the augmentative effect

on RNAII was less. SarT repressed the expression of α-hemolysin, as determined by Northern blotting, Western blotting, and a rabbit erythrocyte hemolytic assay. This repression was relieved upon complementation. Similar to agr and sarA mutants, which predictably displayed a reduction in hla expression, the agr sarT mutant exhibited a lower level of hla transcription than the sarT mutant. In contrast, hla transcription was enhanced in the sarA sarT mutant compared with the single sarA mutant. Collectively, these results indicated that the sara locus, contrary to the regulatory action of agr, induced α-hemolysin production by repressing sarT, a repressor of hla transcription.

L43 ANSWER 32 OF 32 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-021938 [02] WPIDS
DOC. NO. CPI: C2000-005217
TITLE: New accessory regulatory protein, sar, from **Staphylococcus aureus**, used to design analogs potentially useful as antibacterial agents.
DERWENT CLASS: B04 D16
INVENTOR(S): CHEUNG, A; FISCHETTI, V A
PATENT ASSIGNEE(S): (SIGA-N) SIGA PHARM INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5976792	A	19991102	(200002)*		30

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 5976792	A	CIP of	US 1994-248505	19940524
			US 1996-676782	19960708

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 5976792	A	CIP of	US 5587288

PRIORITY APPLN. INFO: US 1996-676782 19960708; US 1994-248505 19940524
AB US 5976792 A UPAB: 20000112
NOVELTY - Isolated, purified and full-length **Staphylococcus aureus** accessory regulatory protein ((I), designated sar) which regulates the expression of S. aureus exoprotein **virulence** determinants (EVD) and has a sequence of about 124 amino acids (aa) as given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) fragments of (I), i.e. the **sarA**, ORF3 or sart proteins;
(2) isolated and purified DNA (II) that encodes (I);
(3) replicable expression vector containing (II);
(4) isolated antibodies (Ab) directed against (I), optionally linked to a reporter molecule;
(5) **sarA** proteins of molecular weight 14.7-14.8 kD and isoelectric point about 8.5;
(6) fusions of **sarA** with a heterologous protein;
(7) purified DNA (IIa) that encodes **sarA**;
(8) replicable expression vector containing (IIa); and
(9) method for detecting the sar gene in a microbial isolate by

testing its DNA with a labeled (II)- or (IIa)-based probe.

ACTIVITY - None given.

MECHANISM OF ACTION - (I) controls the expression of **virulence** determinants such as endotoxins in S. aureus.

USE - (I) is used to design analogs that interfere with expression of EVD, i.e. potential antibacterial agents and for generating specific antibodies which are used to detect (I) in microbial isolates or for affinity purification of (I). The nucleic acid (II) that encodes (I) (or its fragments) can be used to identify S. aureus that express sar (and thus EVD) by usual hybridization and amplification tests, also for recombinant production of (I).

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